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14. ABSTRACT Telomeres, the protective elements at the ends of chromosomes, must be maintained for cells to proliferate indefinitely. In many human cancers the telomeric DNA is replenished by the enzyme telomerase. However, a second pathway for telomere maintenance, referred to as the ALT pathway, has increasingly been recognized in human cancers. The genetic basis for activation of ALT is not known, but recent data have identified mutations and loss of ATRX protein as being hallmarks of ALT-immortalized cell lines and tumors. Our efforts to understand the mechanism by which loss of ATRX facilitates telomere recombination have allowed us to exclude telomeric deposition of the histone variant H3.3, changes in subtelomeric methylation, as well as changes in telomeric nucleosomal organization as being relevant mechanisms contributing to this phenotype. I have also determined that ATRX does not function in the known pathways of HDR repression at telomeres. Instead, I find that loss of ATRX produces a telomere-specific defect in chromosome cohesion, thus identifying a potential mechanism through which loss of ATRX could be important for ALT-mediated telomere maintenance.					
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Table of Contents

	<u>Page</u>
Introduction.....	2
Body.....	3
Key Research Accomplishments.....	9
Reportable Outcomes.....	10
Conclusions.....	10
References.....	11
Supporting Data.....	14

Introduction

Human cancer cells require a telomere maintenance mechanism for their unlimited proliferative potential. Most achieve this by up-regulating telomerase, an enzyme that catalyzes the de novo addition of telomeric repeats to the end of chromosomes^{1,2,3}. A significant minority of tumors activates a telomerase-independent mechanism referred to as alternative lengthening of telomeres (ALT)^{4,5}. The characterization of ALT has remained largely descriptive since this mechanism was identified, and the recurring features of long and heterogeneous telomeres, extrachromosomal telomeric circles, and ALT-associated PML bodies (APBs) have been the basis for attempts to identify tumors reliant on ALT^{4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14}. However, it is unclear whether these characteristics are relevant for the ALT mechanism of telomere maintenance or sufficient for properly identifying tumors reliant on ALT.

Several important observations have provided insight into the mechanism of telomere maintenance in ALT cells. Cell fusion experiments that rescued the ALT phenotype suggested ALT might result from recessive mutations and loss of a normal function¹⁵. The requirement for mutational alterations is also consistent with the low frequency of ALT induction in vitro. Additionally, multiple lines of evidence suggest that despite the repression of homology-directed repair (HDR) at functional telomeres, ALT cells maintain their telomeres by a telomere-specific recombination mechanism^{16, 17, 18, 19}.

The genetic requirements for the activation of telomere recombination and ALT are largely unknown, but recent work by us and others have identified mutations and loss of the ATRX protein as being hallmarks of ALT-immortalized cell lines and tumors^{20,21}. ATRX is a member of the SNF2 family of chromatin remodeling proteins possessing a helicase/ATPase domain. It cooperates with DAXX to deposit the histone variant H3.3 at telomeres and other G-rich repetitive sequences in a replication-independent chromatin assembly pathway^{22, 23, 24, 25}. The function of ATRX/DAXX and H3.3 at telomeres is unclear, but loss of ATRX is associated with reduced telomeric loading of HP1 α , an upregulation of TERRA, and telomere dysfunction in mouse ES cells^{26, 24}. Loss of ATRX also

causes defects in mitotic progression and sister chromatid cohesion, resulting in the formation of lobulated nuclei, micronuclei, and chromatin bridges^{27, 28, 29}.

Our efforts are currently focused on understanding the mechanism by which loss of ATRX facilitates telomere-specific recombination, and determining whether ALT-immortalized cells display specific sensitivities that can lead to more effective treatments for ALT positive cancers.

Body

I first wished to confirm the presence of ATRX at somatic cell telomeres, since much of the localization data has previously been obtained using mouse embryonic stem cells^{24, 26}. Telomeric localization of ATRX was confirmed by ChIP analysis of the endogenous protein in MEFs containing a floxed ATRX allele (Figure 1A). CRE-mediated deletion of ATRX showed an appropriate and significant reduction in the association of the protein with telomeric DNA, confirming the specificity of the interaction (Figures 1A-B). ATRX is less abundant at telomeres than the constitutively bound shelterin protein TRF2. This could partially reflect a transient or cell cycle-regulated association of the protein with telomeric DNA, as has been suggested in mouse ES cells²⁴.

To determine whether the telomeric localization of ATRX was mediated by an interaction with the telomere-specific binding complex shelterin, epitope-tagged proteins (ATRX with TRF1, TRF2, POT1, TPP1, or TIN2) were co-expressed in 293T cells and binding was assessed by co-immunoprecipitation. Under conditions that maintained the interaction between ATRX and its binding partner DAXX, no interaction was observed between ATRX and any shelterin subunit (data not shown). However, DAXX co-precipitates with both TRF1 and TRF2 (Figure 1C), providing one potential mechanism for the recruitment of ATRX to telomeres.

ATRX and DAXX function together as a histone chaperone complex to deposit H3.3 at telomeres and pericentric heterochromatin in a replication-independent chromatin assembly pathway^{22, 23, 24, 25}. A recent study identified H3.3 mutations in a subset of pediatric glioblastoma multiforme tumors predicted

to be reliant on ALT³⁰. This correlation suggested the ATRX-DAXX-H3.3 pathway may be the relevant mechanism through which telomere recombination is unleashed in ALT cells. To test this, we stably expressed an epitope-tagged H3.3 variant in the telomerase-positive HeLa 1.3 cells (a clonal cell line with long telomeres) and in three ATRX-deficient human ALT cell lines. Deposition was then assessed by ChIP using a probe for telomeric DNA, as well as a probe for the repetitive Alu sequences as a positive control. The H3.3 variant was associated with telomeric DNA in the HeLa 1.3 cells and all three ATRX-deficient ALT cell lines (Figures 2A-C). The minor variations observed in telomeric association are likely due to differences in the expression level of H3.3-His between the cell lines. Similarly, deposition of the H3.3 variant at the repetitive Alu elements occurred in both telomerase-positive and ALT cell lines. Furthermore, Cre-mediated deletion of ATRX in MEFs stably expressing epitope-tagged H3.3 did not diminish the amount of telomeric DNA associated with H3.3 (Figures 2D-E). These data demonstrate that telomeric deposition of H3.3 is not perturbed in ALT cells, and additionally that telomere deposition can occur independently of ATRX. The reported reliance on ATRX-DAXX for H3.3 deposition at telomeres may be specific to the mouse ESCs used in that particular study²⁶. Additional experiments to identify the histone chaperone complex responsible for H3.3 localization to telomeres in ALT cells have suggested redundant mechanisms contribute to its deposition (data not shown).

After excluding the histone variant as being the relevant effector in the ATRX pathway contributing to telomere recombination and ALT, I wished to examine whether loss of ATRX itself could influence telomere recombination. I first assessed telomere recombination using the C-circle assay in MEFs before and after CRE-mediated deletion of ATRX. C-circles are extra-chromosomal telomeric DNA circles containing both single-stranded and double-stranded DNA. They are believed to be byproducts of telomere recombination events, and are abundant in and specific to ALT cells^{14,31-33}. C-circles can be efficiently amplified by rolling circle amplification and detected by dot blot using a telomere specific probe¹⁴. I found no change in the abundance of C-circles after deletion of ATRX

(Figures 3A-C). By comparison, the level of C-circles observed in the ALT cell line JFCF6/T.1M was consistently five-fold higher than that observed in the MEFs. This suggests that deletion of ATRX does not enhance telomere recombination events that would generate extra-chromosomal C-circles.

I also examined telomere recombination using the chromosome orientation FISH (CO-FISH) assay, which allows detection of recombination events between sister telomeres (T-SCEs)³⁴. Cells are cultured for one round of replication in the presence of the thymidine analog BrdU, allowing incorporation into the newly synthesized strands. The daughter strands are then removed by nuclease digestion, leaving single-stranded DNA to which telomere strand-specific probes (TelC or TelG) can efficiently hybridize. A chromosome end was considered to be positive for a T-SCE when both chromatids showed hybridization to both the TelC and TelG probes. Two independent MEF cell lines were assessed for T-SCEs after Cre-mediated deletion of ATRX. Both consistently showed T-SCEs at 1-2% of chromosome ends in the presence or absence of ATRX (Figures 3D-F). This level of T-SCEs is the typical background observed in MEF cell lines, and is significantly lower than the ~10% of chromosome ends displaying T-SCEs when HDR repression is perturbed³⁵. These results indicate deletion of ATRX also does not unleash telomere recombination events visible as telomere sister chromatid exchanges.

Since deletion of ATRX alone did not produce a recognizable defect in HDR repression at telomeres, I generated MEFs where both ATRX and the shelterin protein Rap1 could be deleted, or Ku70^{-/-} MEFs in which ATRX could be deleted. The de Lange lab has identified the Ku70/80 heterodimer as an important repressor of HDR at telomeres, as well as the shelterin proteins Rap1 and Pot1³⁵⁻³⁷. The shelterin proteins and Ku function in two independent pathways of HDR repression, and deletion of Rap1 and Ku or deletion of Pot1 and Ku is required to abrogate this repression and unleash telomere recombination events, detectable as T-SCEs via the CO-FISH assay (Figure 4A). If ATRX functions in the Ku mediated pathway of HDR repression at telomeres, we would expect deletion of ATRX and Rap1 or Pot1 would increase T-SCEs.

Alternatively, if ATRX functions in the shelterin-mediated pathway of repression, then deletion of ATRX in the Ku^{-/-} MEFs should unleash telomere recombination. Immunoblots confirm the lack of Ku70 expression, as well as successful Cre-mediated deletion of ATRX and Rap1 in the indicated cell lines (Figures 4B-C). Importantly, there was no significant difference in the percentage of chromosome ends with T-SCEs before and after Cre in the ATRX^FKu70^{-/-} or ATRX^FRap1^{F/F} cell lines (Figure 4D). The positive control, Rap1^{F/F} Ku70^{-/-} cells, did show an appropriate increase in telomere recombination after Cre-mediated deletion of Rap1. These results indicate ATRX does not function in either known pathway of HDR repression at telomeres.

Given the absence of a dramatic telomere phenotype after loss of ATRX alone or in combination with proteins known to be important for HDR repression at telomeres, we began to examine additional phenotypes associated with loss of ATRX that could potentially contribute to telomere dysfunction. ATRX patient cells show changes in the patterns of DNA methylation at several highly repetitive sequences³⁸. Ribosomal DNA repeats were hypomethylated while a Y-specific satellite was hypermethylated. Additionally, there were subtle changes observed with a subtelomeric probe, but it was not determined whether this was due to hypomethylation or hypermethylation of the sequences. These changes in subtelomeric methylation could potentially be important for telomere recombination events. Studies in mouse cells have revealed that a lack of DNA or histone methyltransferases alters the methylation status of subtelomeric regions and leads to telomere elongation and increased T-SCEs^{39,40}. Furthermore, the progressive telomere shortening observed in telomerase null mice results in decreased subtelomeric DNA methylation and an increased frequency of telomere recombination⁴⁰. We therefore examined subtelomeric methylation in both ATRX-deficient ALT cell lines and in the ATRX flox MEFs using methylation-sensitive restriction endonucleases. MspI and HpaII are isochizomers with different sensitivities to CpG methylation. HpaII digestion is blocked by CpG methylation where as MspI is not. Genomic DNA was isolated from telomerase-positive BJ hTERT SV40 cells and from the twenty-three ALT

cell lines we have available (a subset of the ALT cell lines is shown). The DNA was digested overnight with MspI or HpaII and the samples were separated by pulse-field gel electrophoresis. Ethidium bromide staining was used to detect the bulk genomic DNA while telomere fragments were detected by Southern blot using a ^{32}P -labelled telomere probe. MspI will digest the DNA and release the telomeric fragments irrespective of the subtelomeric methylation status. However, the presence of CpG methylation will block digestion by HpaII and restrict its activity to regions farther from the chromosome end, producing longer telomere fragments and an observed shift in the mobility of those fragments. Low levels of subtelomeric methylation would be expected to produce telomere fragments of a similar size to those released by MspI. A slower migrating band is observed in the telomere blot for the BJ hTERT SV40 cells digested with HpaII compared to the MspI digest, indicating the presence of subtelomeric methylation (Figure 5A). The interpretation of the ALT digests is more difficult because the telomeres are very heterogenous in length. The obvious shift in mobility is not observed, but a subset of the ATRX-deficient ALT cells display an increased intensity of the higher molecular weight telomere fragments with HpaII digest (Figure 5A). This may indicate a low level of CpG methylation in the ALT cell lines, suggesting these subtelomeric regions are hypomethylated relative to the telomerase positive control. Another study also identified differences in subtelomeric methylation among different ALT cell lines, but did not observe any correlation between the subtelomeric methylation status and the level of T-SCEs⁴¹. Furthermore, I observed no change in the pattern of restriction digest after Cre-mediated deletion of ATRX in MEFs (Figures 5B-C). This suggests that loss of ATRX does not produce a dramatic change in subtelomeric methylation, and likely excludes changes in subtelomeric methylation as a potential contributor to the telomere recombination that is characteristic of ALT.

Members of the SNF2 family of chromatin remodeling proteins, such as ATRX, can use ATP hydrolysis to translocate nucleosomes in cis along the DNA⁴². Recent work from David Allis' lab has shown the ATRX-DAXX complex can catalyze the formation of extended nucleosome arrays and effectively

mobilize a nucleosome along template DNA²⁵. ATRX could thus function to suppress repair events at telomeres by altering nucleosome positioning, with loss of ATRX causing changes to nucleosome mobility that may allow access by repair proteins. I therefore examined the telomeric nucleosomal organization before and after deletion of ATRX. Nuclei were digested with increasing amounts of MNase, and the resulting products were fractionated on an agarose gel. Bulk chromatin was visualized by ethidium bromide staining, followed by detection of telomeric nucleosomes by Southern blot hybridization with a ³²P-labelled telomeric probe. Both the bulk and telomeric chromatin show no obvious change in the MNase sensitivity or periodicity of the nucleosomal ladder after deletion of ATRX (Figures 6A-B). Therefore, loss of ATRX does not likely promote telomere recombination by altering telomere nucleosomal organization.

Loss of ATRX has also been reported to cause a delay in mitotic progression, and is associated with defects in sister chromatid cohesion and congression at the metaphase plate²⁹. In addition, ATRX co-immunoprecipitates with components of the cohesin ring complex⁴³. I became interested in the cohesion function of ATRX upon recognizing the high degree of overlap between the phenotypes of defective chromosome cohesion and those of ATRX loss. Both show inaccurate chromosome segregation, an inability to maintain the processivity and stability of replication forks, an increased sensitivity to replication inhibitors, as well as changes in gene transcription^{23,29,44-48}. Thus, many of the phenotypes associated with ATRX deficiency could potentially result from chromosome cohesion defects. And importantly, there is a telomere-specific cohesin complex (containing SA1 instead of SA2), potentially allowing for differential regulation of telomere cohesion versus arm cohesion⁴⁹. Reports in the literature also appear to suggest T-SCEs are elevated under conditions where there are defects in both replication fork processivity and stability, known consequences of cohesion defects^{50, 51, 52}. The collective data indicates cohesion is important for the alignment of sister chromatids, and would preferentially promote repair on the sister chromatid as a template. Additionally, by keeping the sister chromatids in register, proper cohesion establishment would minimize

any changes in length due to homologous recombination events. My hypothesis is that ATRX deficiency may produce a telomere-specific cohesion defect. This would allow precocious separation of sister chromatids, resulting in impaired replication. The dissociation of the sister chromatids would allow repair of stalled replication forks to take place using non-sister telomeres, thereby abolishing the restriction on both template use and register. This proposed mechanism could provide an explanation for the observed changes in telomere length associated with ALT, as well as the recombination events. Of note, is that T-SCEs may be an underrepresentation of the amount of telomere recombination occurring under these conditions. Because repair events could occur with any other chromatid, unequal exchanges may result in an abnormally short or long telomere at one chromatid (while maintaining the correct telomere strand sequence), in addition to the strand exchanges scored as T-SCEs.

To assess telomere and arm cohesion, I performed FISH on interphase cells using probes for the subtelomeric and arm regions of mouse chromosome 10. After confirming the appropriate localization of the probes on metaphase spreads (Figure 7A), FISH signals in interphase cells were scored as either a single focus or a doublet (indicative of premature separation and thus a cohesion defect⁴⁹). Results from three independent experiments confirm a significant increase in the percentage of telomere signals as doublets after Cre-mediated deletion of ATRX (Figure 7B-C). The loss of ATRX had no effect on the percentage of arm signals observed as doublets. This suggests ATRX is important specifically for telomere cohesion, and identifies a potential mechanism through which loss of ATRX could be important for ALT-mediated telomere maintenance.

Key Research Accomplishments

- Exclusion of the ATRX-DAXX-H3.3 pathway as being the relevant mechanism through which telomere recombination is unleashed in ALT cells
- Identification of a telomere-specific cohesion defect in cells lacking ATRX

Reportable Outcomes

- Poster presentation of the described data (excluding Figure 7 due to the highly competitive nature of this project)
 - Cold Spring Harbor Laboratory, Telomeres & Telomerase Meeting (May 2013)

Conclusions

I have excluded numerous functions of ATRX as being relevant for the ALT mechanism of telomere maintenance, and completed all objectives outlined in *Task 1* of the revised SOW. Importantly, deposition of the histone variant H3.3 at telomeres is not perturbed in ATRX-deficient ALT cell lines or in ATRX-deficient MEFs. This indicates the ATRX-DAXX-H3.3 pathway is not the likely effector of the telomere recombination phenotype characteristic of ALT cells. I can further exclude the possibility that ATRX functions in either known pathway of HDR suppression at telomeres, because telomere recombination was not unleashed by the combined loss of ATRX and Ku70 or Rap1. I believe the relevant function of ATRX is to promote telomere-specific chromosome cohesion. The data demonstrates a significant telomere-specific cohesion defect after loss of ATRX. Cohesion defects are expected to produce problems with replication fork processivity and stability. This would be exemplified by the presence of fragile telomeres on metaphase spreads⁵⁰, which I intend to evaluate in both ALT cell lines and the ATRX-deficient MEFs. Furthermore, the lack of cohesion is expected to abrogate the preferential use of the sister chromatid for repair, potentially resulting in unequal exchange events that may be detected on metaphase spreads via the CO-FISH or quantitative FISH assays. To ensure this phenotype is not masked by the presence of telomerase in the MEFs, new ATRX^F cell lines will be created in a telomerase-deficient background (mTR^{-/-}). I plan to confirm the telomere-specific cohesion defect with FISH probes for additional chromosomes. I am also interested in determining whether general cohesion defects can promote telomere recombination events, and in identifying the precise role ATRX plays in establishing telomere cohesion. *Tasks 2 and 3*

are still important undertakings. I hope to continue my progress on the revised SOW while examining the role ATRX plays in chromosome cohesion and the ALT mechanism of telomere maintenance. Although ATRX mutations may be a useful indicator of the presence of ALT in cells and tumors, it is critical to identify any chemotherapeutic sensitivities these cells may have such that better treatment options can be employed.

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Supporting Data

Figure 1. ATRX is associated with telomeric DNA in somatic cells. **A.** Top, telomeric ChIP for ATRX in two independent ATRX^{Flox} MEF lines. Cells were fixed and analyzed 96 hr after Cre infection. Pre-immune serum was used as a negative control, and immunoprecipitation of the shelterin protein TRF2 was used as a positive control. Bottom, immunoblot for ATRX and γ tubulin (loading control) from total cell extracts of the indicated ChIP samples. **B.** Quantitation of the ChIP data shown in A. Bars indicate the average and standard deviation of three independent experiments. The –CRE samples were used as reference values (ref) and set to 100%. **C.** Co-immunoprecipitation of DAXX with TRF1 and TRF2 from 293T cells transfected with Flag-DAXX and myc-tagged TRF1 or TRF2. Flag IPs were immunoblotted with Flag and myc antibodies. As a positive control, co-immunoprecipitation of DAXX with ATRX was observed in 293T cells transfected with Flag-DAXX and ATRX-HA under the same conditions.

Figure 2. Telomeric deposition of H3.3 is not disrupted in ALT cells or after deletion of ATRX. **A.** Telomeric ChIP (top) and Alu repeat ChIP (bottom) for H3.3-His in the telomerase-positive HeLa 1.3 cells and three ATRX-deficient ALT cell lines. Pre-immune serum and TRF2 were again used as negative and positive controls, respectively. **B.** Quantitation of the ChIP data shown in A. Bars represent the average and standard deviation of three independent experiments. The HeLa 1.3 samples were used as reference values (ref) and set to 100%. **C.** Immunoblot for H3.3-His and γ tubulin (loading control) from total cell extracts of the indicated samples. **D.** Top, telomeric ChIP for H3.3-myc in two independent ATRX^{Flox} MEF lines stably expressing the epitope-tagged protein. Pre-immune serum and TRF2 were again used as negative and positive controls, respectively. Bottom, immunoblot for ATRX, H3.3-myc, and γ tubulin from total cell extracts of the indicated ChIP samples. **E.** Quantitation of the ChIP data shown in D. Bars indicate the average and standard deviation of three independent experiments. The –CRE samples expressing H3.3-myc were used as reference values (ref) and set to 100%.

Figure 3. Deletion of ATRX does not enhance specific telomere recombination events. **A.** Immunoblot of ATRX in two independent ATRX^{Flox} MEF lines before and 96hr after Cre infection. Actin was blotted as a loading control. **B.** C-circle assay in ATRX^{Flox} MEF lines shown in A. The ALT cell line JFCF6/T.1M was included as a positive control. **C.** Quantitation of three independent C-circle experiments, with the average and standard deviation shown. The JFCF6/T.1F sample was used as a reference value (ref) and set to 100. **D.** Immunoblot of ATRX in two independent ATRX^{Flox} MEF lines before and 96hr after Cre infection. The γ tubulin blot was included as a loading control. **E.** Representative images of CO-FISH staining in ATRX^{Flox} MEFs before and after Cre-mediated deletion of ATRX. Fluorescence signals of the TelG and TelC probes are depicted separately and merged with DAPI. **F.** Quantitation of

COFISH experiments in the ATRX^{Flox} MEF cells lines. The bars depict the average and standard deviation of three independent experiments.

Figure 4. ATRX does not function in either known pathway of HDR repression at telomeres. **A.** The six subunit shelterin complex bound to telomeric DNA is shown. The shelterin proteins Rap1 and Pot1a/b are responsible for inhibition of telomeric HDR. In a separate pathway is a general repressor of HDR, the Ku70/80 heterodimer. Inhibition of both pathways is required to unleash HDR events at telomeres. ATRX could function in the shelterin- or Ku-mediated pathways, or possibly an independent mechanism of HDR suppression at telomeres. **B.** Immunoblot for ATRX, Ku70, and Rap1 in ATRX^{Flox} and two independent ATRX^{Flox} Ku70^{-/-} MEF cell lines used for CO-FISH analysis, before or 96hr after Cre infection. The cell lines have no detectable Ku70 protein, and Cre-mediated deletion of ATRX successfully reduced target protein levels. **C.** Immunoblot for ATRX, Ku70, and Rap1 in ATRX^{Flox} and two independent ATRX^{Flox} Rap1^{F/F} MEF lines used for CO-FISH analysis. The cell lines show successful reduction of both ATRX and Rap1 after Cre infection. **D.** Quantitation of CO-FISH experiments for the cell lines depicted in B and C. The bars indicate the average and standard deviation of samples from three independent experiments.

Figure 5. Loss of ATRX does not dramatically alter levels of subtelomeric methylation. **A.** Genomic DNA from BJ hTERT SV40 or ALT cell lines was digested with MspI (M) or HpaI (H) and equivalent amounts of DNA were separated on a 1% agarose gel using pulse-field gel electrophoresis. Total DNA was stained with ethidium bromide (left) and telomeric DNA was detected by Southern blot using a ³²P-labelled telomere probe (right). **B.** Genomic DNA was isolated from ATRX^{Flox} MEFs before and 96hr after Cre infection. DNA was digested with AluI+MboI (A), MspI (M), or HpaI (H) and equivalent amounts of DNA were separated by pulse-field gel electrophoresis. Total DNA was stained with ethidium bromide (left) and telomeric DNA was again detected by Southern blot (right). **C.** Immunoblot for ATRX in the ATRX^{Flox} MEFs used for analysis of subtelomeric methylation. A successful reduction in ATRX protein is observed at 96hr after Cre infection, and the γ tubulin blot indicates equal loading of the samples.

Figure 6. Loss of ATRX does not alter bulk or telomeric nucleosomal organization. **A.** Nuclei from ATRX^{Flox} MEFs before and 96hr after Cre infection were incubated with increasing amounts of MNase and fractionated on a 1.5% agarose gel. Bulk nucleosomes were detected by ethidium bromide staining (left) and telomeric nucleosomes were detected by Southern blot hybridization with a ³²P-labelled telomeric probe. **B.** Immunoblot confirming loss of ATRX protein in the Cre-treated ATRX^{Flox} MEFs.

Figure 7. Loss of ATRX produces a telomere-specific cohesion defect. A. Metaphase spread showing appropriate hybridization of the FITC-labeled subtelomeric probe and the rhodamine-labeled arm probe. The adjacent chromosome ideogram indicates the approximate location of the two probes used. **B.** FISH analysis of ATRX^{Flox} MEFs in interphase before and 96hr after Cre infection, with probes for the subtelomeric and arm regions of chromosome 10. **C.** Quantitation of the FISH signals observed as doublets for the arm and telomere probes before and after deletion of ATRX. Approximately 200 FISH signals for each probe were analyzed from three independent experiments. *** indicates $p < 0.0005$.

Figure 1

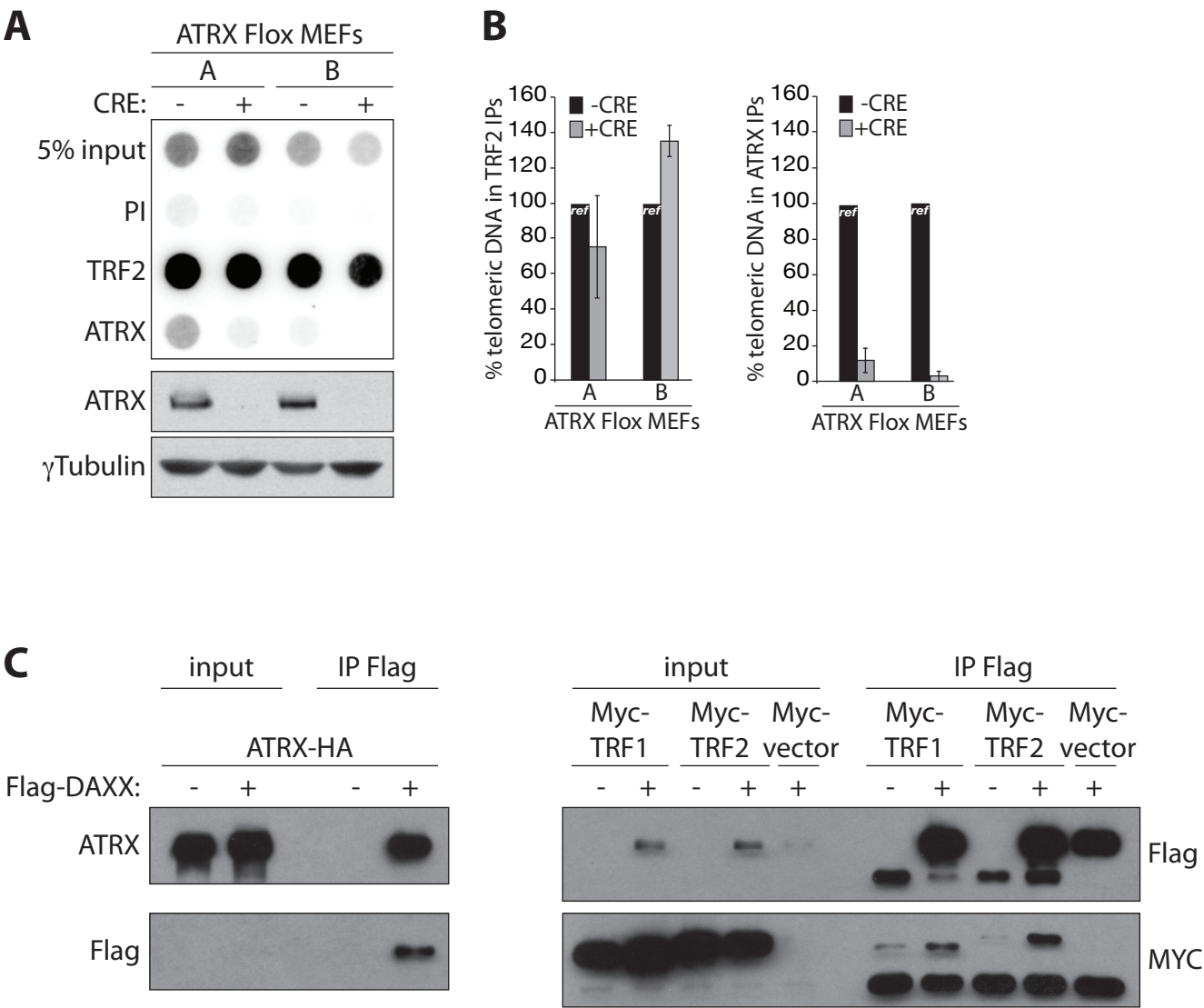


Figure 2

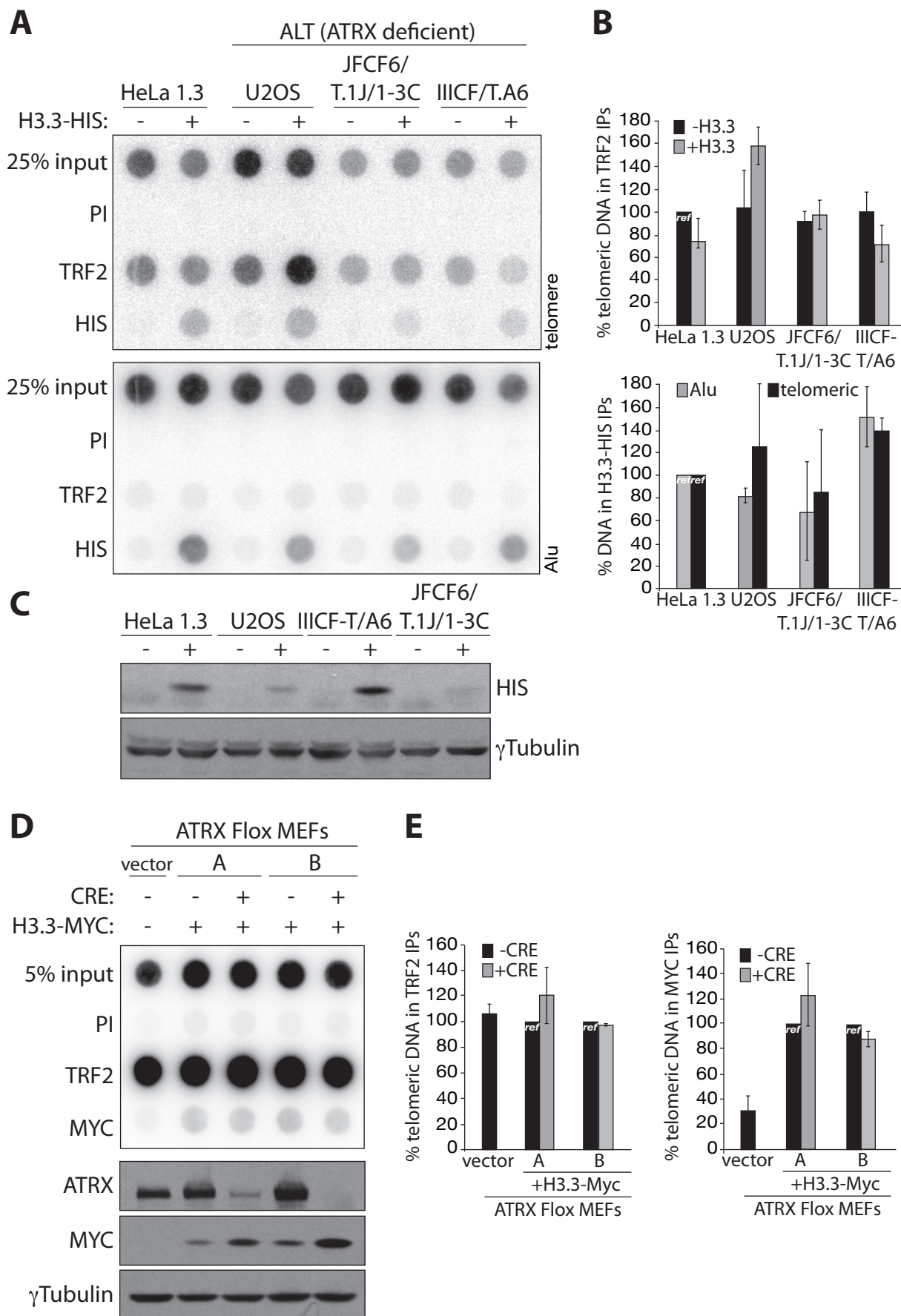
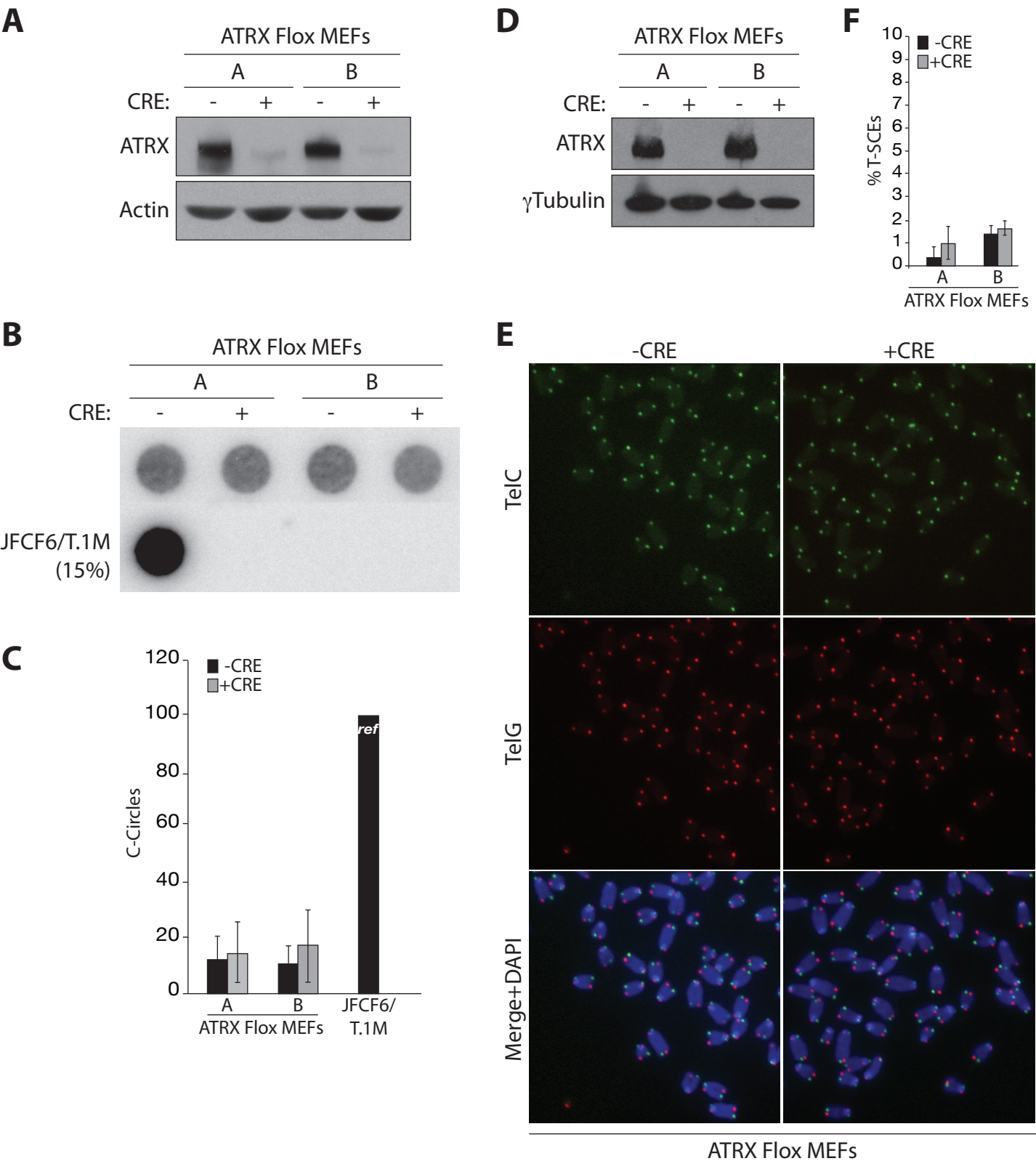


Figure 3



A

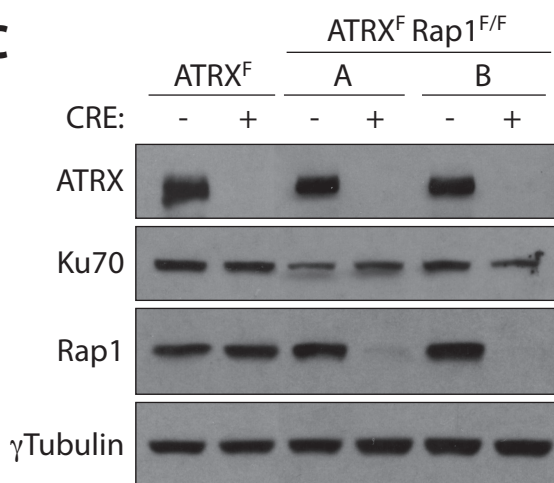


Figure 5

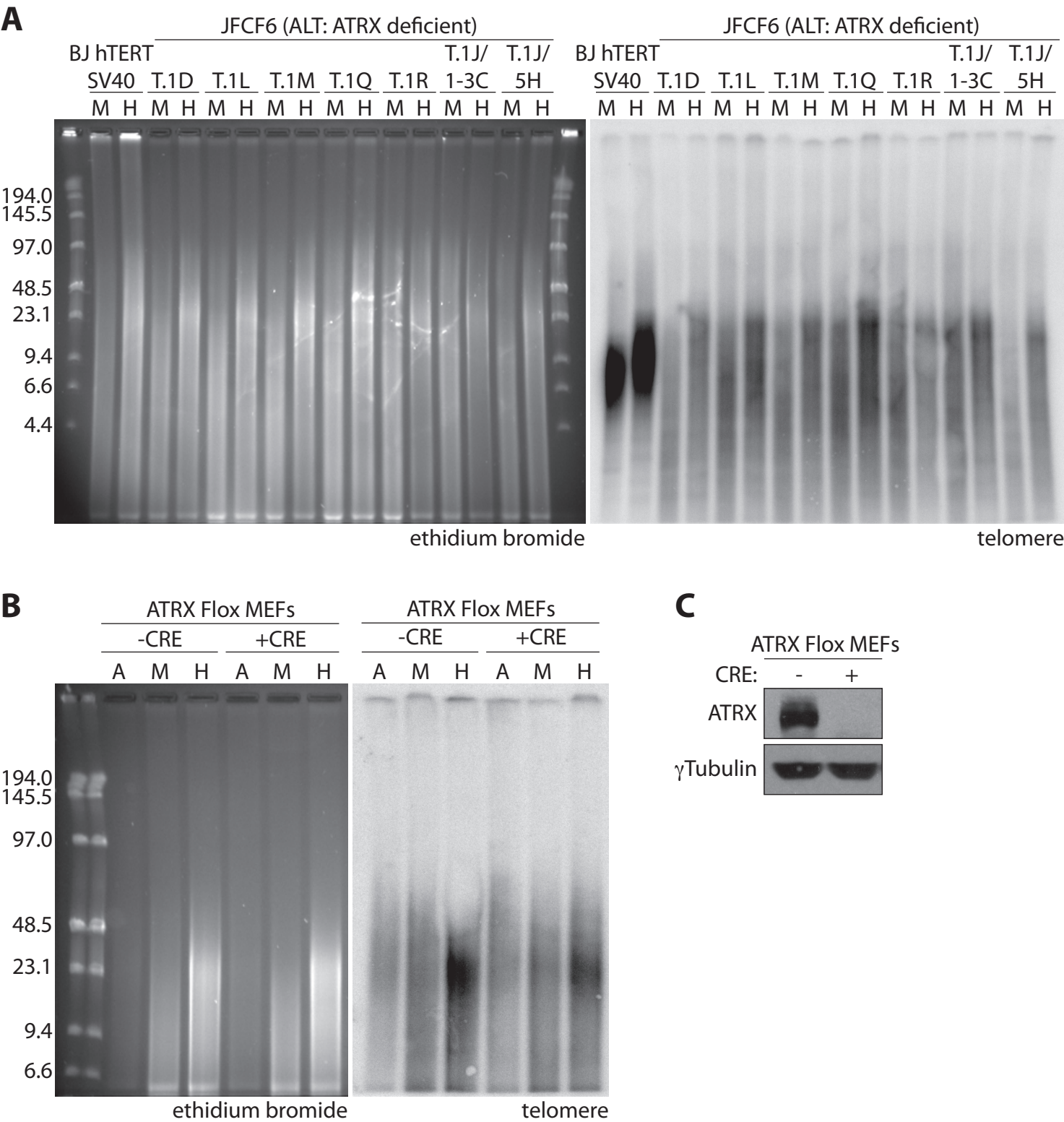


Figure 6

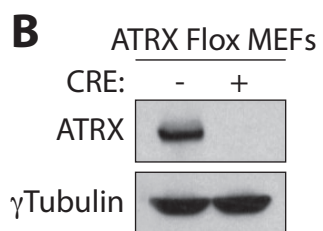
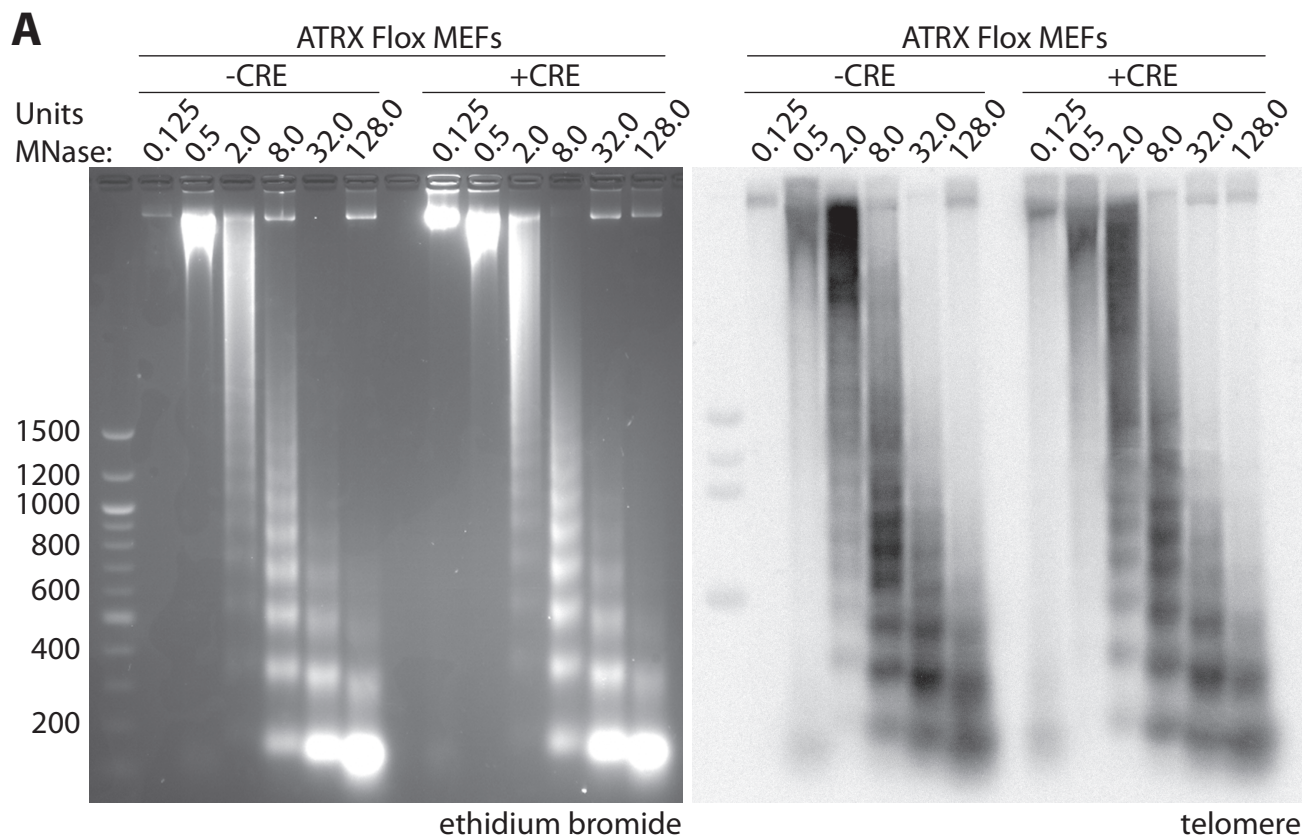


Figure 7

